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Synopsis

Thesis Title: High moisture extrusion of soy protein and millet flour blends for development of texturized protein rich foods

Protein deficiency, a deficit in the quantity and quality of protein in diet, is a major public nutritional and health problem in many developing countries especially in India. Beside low intake of proteins, a significant factor contributing to widespread protein deficiency in India is that a large portion of India's dietary protein comes from plant based foods which have low amino acid scores in general. Thus, quality of protein is intertwined. Additionally, the plant proteins coming primarily from cereals and pulses in the diet, bring along a high carbohydrate and phytate load at intakes required to achieve protein sufficiency. This potentially leads to susceptibility to metabolic syndrome and mineral nutrient deficiency. Transitioning to a diet predominant in diary and meat is culturally unlikely, but also environmentally a poor choice. Going forward the world population is expected to reach 10 billion people by 2050 with India accounting for nearly 2 billion. Since meat and dairy proteins have a much higher environmental footprint, it is desirable that the growing protein needs of humanity are met through plant proteins. A potential option that meets both nutritional and environmental challenges is the use of blends of plant protein isolates that combine legumes and cereals to obtain high amino acid scores and digestibility. Further, extrusion of these protein rich blends creates novel textures.

Expanded 'non-anisotropic' structured proteins made through low moisture extrusion have been available in the market for few decades. However, their consumer uptake has been low. A major reason is texture. The products are expanded during extrusion and lead to light, spongy, non-anisotropic texture. Developing products that can extend the textural range by increasing density, fibrousity and anisotropy may help product developers craft new consumer opportunities. In this thesis plant protein based products have been developed and characterized that have dense and anisotropic textures with a good amino acid profile using blends of soy protein isolate and minimally processed millet flour and the fractions in a high moisture extrusion process using a twin screw extruder. These can be used for many applications such as soy chap and meat analogues.

The physicochemical and nutritional characteristics of plant protein isolates of soy, pea, chickpea, rice and wheat, were analyzed in the laboratory as these isolates are commonly used for making texturized plant protein rich foods. The results are presented in **Chapter 3** and show significant differences from each other. Together, water absorption capacity and water solubility of a protein are key determinants of the textural quality of their extrudates. The fibrous textures are a result of the difference in values of these properties between the key components of the blends used for extrusion. Generally, the greater the difference the more the fiber density. Soy isolate used in this study had a water absorption capacity of more than 5 g/g protein, while wheat gluten could absorb around 2 g/g. PPI, CPP and RPI had water absorption capacities in between SPI and WG. When it came to water solubility, chickpea isolate had the highest WS whereas, WG and RPI the lowest. SPI and PPI had intermediate solubilities. Thus, SPI provides the best physicochemical characteristics for high moisture extrusion. It has a high water absorption capacity and moderate solubility. It is suitable for blending with other materials with properties very different from itself, such as millet flours which have been used in this work) to give good fibrous textures.

Another important consideration for high moisture extrusion is the least gelling concentration. Gelling plays a key role in the cooling die. SPI gelled at 12% while the gelling concentration was 14% for PPI, 16% for CPP and 20% for RPI. While WG also gelled at 12% concentration, due to its low water absorption capacity its gels released a lot of free water.

A few researchers suggest the use of oil in the blends during extrusion. While this was outside the scope of experiments in this work, if oil has to be included in the blends during extrusion, then having some emulsion capacity is important. SPI, PPI and CPP had higher emulsion capacities and would be more suitable for this compared to RPI and WG which have poor emulsion capacities.

Pasting properties of the protein powders give an indication of the melt viscosities. SPI had the highest final viscosity and RPI the least with the descending order being SPI > PPI > CPP > WG > RPI. Rheological characteristics of the powders give even a better idea of how the melts behave under extrusion conditions. Interestingly the descending order of complex viscosity for the powders is SPI > CPP > RPI > WG > PPI, which is different from the order based on the pasting curves.

There are a number of parameters that effect the textural quality of protein extrudates as described in **Chapter 4**. In this thesis we divide them into raw material parameters, system parameters, process parameters and product properties. The main raw material parameter is the protein concentration of the feed. The process parameters include feed moisture content, barrel temperature, screw speed and feed rate. The system parameters include the specific mechanical energy, melt temperature and melt pressure. The effect of protein concentration and the process parameters on the textural quality was studied for soy proteins. Protein concentration of the blend (dry basis) and moisture were found to be the most important parameter. Barrel temperature profile also had a significant impact on the texture of the extrudates. The other parameters, namely screw speed and feed rate, had smaller effects and could be used for finer modulation of texture.

Textural parameters such as hardness correlated linearly with protein concentration of wet basis. By changing protein concentration and process parameters, a wide range of hardness (11X) and chewiness (9.6X) was created. The range in values of resilience (2.2X), cohesiveness (1.4X) and springiness (1.2X) was much smaller than hardness and chewiness. Hardness and chewiness of real meat were in the range of HMMA obtained by levering process parameters. Other parameters (resilience, cohesiveness & springiness) of HMMA were out of the range obtained (higher values than real meat). A part of this gap was bridged using millet flours as blend components.

In **Chapter 5**, three types of millet - sorghum, pearl millet and finger millet flours were used as secondary components, each blended separately with soy protein isolate. The addition of millets reduced the hardness and chewiness of millets while at the same time showing improved visual fibers. This was shown from incorporation levels of millet flours up to 30%. Each millet had a slightly different effect on the texture and colour of the extrudates. Sorghum and pearl millet blends had thicker fibers and whiter in colour while finger millet blends had finer fibers and reddish brown in colour. Comparative texture profile analysis between extrudates and real meat (chicken breast) revealed that all millet blends brought the textural values closer to those of real meat. Notably, finger millet significantly shifted the texture band towards the real meat profile. Besides providing positive changes in texture and providing a natural colour pallet, incorporation of millets also improved the amino acid profiles of the extrudates. SPI is low on sulphur containing amino acids (SAA) while millets lack lysine. Blends of SPI and millet flours at 30% showed balanced amino acid profile, good amino acid score and equivalent protein content compared to real meat, though it reduces the overall protein content marginally.

Compared to SPI and wheat gluten blends, which are the most common blends used in industry, the SPI and millet flour blends have a better amino acid score. This is because wheat gluten is low on lysine and incorporation of wheat gluten at high levels of 30% negatively impacts the amino acid scores of the extrudates significantly. Additionally, millets are gluten free and therefore the SPI and millet flour blends are suitable for a population that is gluten intolerant.

In **Chapter 6**, one of the millets, finger millet, was fractionated by separating the hull. The flour obtained from the hull and the dehulled grains were characterized. The hull flour was found to be rich in fiber and the dehulled flour was found to be low in fiber. Its starch content was much higher than the hull fraction. Therefore, the hull fraction was called fiber rich flour or FRF and the dehulled fraction was named starch rich flour or SRF. FRF had 9.4 times the fiber compared to SRF while SRF contained 1.7 times the amylopectin compared to FRF.

SPI was blended with SRF and FRF and extruded. All extrudates displayed visual fibration. However, they differed in the fiber density, that is, the number of fibers per unit cross section length. SPI100% showed the least fiber density while SRF and FRF blends showed a high density of fibers. Among the blends, SRF had the thicker and longer fibers and FRF had the thinner and shorter fibers. However visual anisotropy did not translate into cutting strength anisotropy. Cutting strength anisotropy was maximum for SRF blends and minimum for SPI100%. Extrudates from blends had a textural profile closer to real meat compared to SPI100% extrudates. SRF was closest among different blends. Extrudates from FRF blends were more resilient than those from SPI100%; SRF blends were less resilient.

A mechanistic approach was used to understand the structure from a protein interaction in different solvents. Protein solubility in phosphate buffer showed least solubility and all extrudates showed comparatively lower protein solubility than non-extruded materials, suggesting native proteins were aggregated during the high moisture extrusion. The presence of higher hydrogen and disulfide bonds in SRF blend extrudates probably die to the higher starch/amylopectin content and resulting in a higher anisotropic index. Higher hydrophobic interactions in FRF blend extrudates was mostly due to the insoluble dietary fiber which contains hydrophobic units. Other factors for the harder structure of FRF blend extrudates could be due to the presence of Ca^{2+}in FRF.

An image analysis method was developed to quantify the features of the fibers in the extrudates. This straightforward tool assists researchers and manufacturers in quantitatively characterizing visual fibrations.

The key takeaway from the study is the significance of aligning the properties of the protein and minimally processed flours/fractions for successful structure formation to create desired textures for masses with good nutritional profile. The integration of millets into blends, represents a paradigm shift in the development of protein rich texturized products. As the demand for sustainable and affordable texturized plant based foods continues to grow, millets stand as an ingredient and the way of minimally processed fractions as an innovative approach, offering a path to meet the expectations of texture and environmental impact.